Application of PBTK/TD Models in Assessing Neurodevelopmental Toxicity in Susceptible Populations

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Science Question

Can physiologically based toxicokinetic/ toxicodynamic (PBTK/TD) models quantitatively address critical issues in developmental risk assessment, such as the identification of windows of susceptibility, inter-and intraspecies variability, and the use of mechanistic/mode of toxicity data?

Observations at various levels of biological complexity (i.e., cell, tissue, organ, organism) can be linked by dose, time, location, and magnitude of response by applying PBTK/TD models. Such models can more effectively address inherent uncertainties associated with dose extrapolations from animals to humans and from adults to children.

Research Approach

- Published toxicokinetic data were collected on MeHg and EtOH distributions in rodent during early organogenesis, and used to develop robust estimates of partitioning between maternal and embryonic tissues.

- The data on chemical disposition were combined with the pregnancy kinetics model to develop a toxicokinetic model of distribution in the pregnant rodent and embryo.

Step 1: Developing TK Models

The kinetics of neurotoxicants in the fetus are defined by a physiologically based TK model to relate observed retention patterns of a toxicant and predict tissue-specific concentrations across times. However, the model of the fetus differs in several important ways from that of the mother. First, the fetus is still developing; hence, all the tissue weights should be treated as a function of time. Second, the physiology of the fetus changes with time; therefore, the blood flow and partition coefficients are time-dependent functions. Third, the metabolism rates often differ between mother and fetus; hence, different lifestage-specific values for V_{max} and possibly K_M are needed.

Step 2: Developing TD Models

Biologically based dose-response (BBDR) models were previously developed for MeHg and EtOH (Lewandowski et al., 2002; Gohlke et al., 2002) to assess their potential impacts on critical neurodevelopmental processes, such as proliferation, differentiation, and migration. These models were derived from a Leroux et al. (1996) TD model that was constructed with in vitro data collected from embryonic midbrain cell cultures. However, the TD model was used in the present study with in vivo data for specific lifestages.

Kinetic and Dynamic Factors of Model

In the TD part of the original Leroux model, the progenitor X cells can potentially divide, differentiate into Y cells, or die. Y cells can either divide or die. However, in the current neocortical model, the Y division rate is set to zero, as Y cells are defined as postmitotic neurons in the cortical plate. Two major assumptions of the underlying mathematical construct are: 1) Differentiation from progenitor X cells to Y cells is irreversible; and 2) Cells act independently of each other. This dynamic model was used to describe normal development in the midbrain and neocortex by applying lifestage-specific parameters.

Step 3: Approach for Linking TK/TD Models

A stepwise, linked TK and TD modeling approach for in utero exposure to the potential effects of neurodevelopmental toxicants was developed in this study. Specifically, the TK part was linked with the Leroux et al. (1996) model output, such that organ dose was calculated at various times throughout the stages of development.

### Leroux Model with Dose Rates
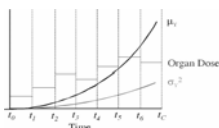
A piece-wise constant approach linked the TK and TD models by taking the output of the TK model (dose at time t) and holding it constant for a given amount of time t in the TD model. Hence, the TD model calculated output (cell number) in steps across developmental time, with each time-step having a constant dose, based on the output from the TK model.

MeHg Case Study

A study was conducted on the response of linked TK/TD model (with in vivo MeHg data) to fetal brain dosing patterns. The fetal brain MeHg concentration was predicted by the TK component of the model and varied over time according to exposure and absorption of the compound. The study focused on midbrain development as an early window of susceptibility to exposure by predicting the effects on midbrain cell number after exposure to maternal doses of MeHg on gestational day (GD)12 (Lewandowski et al., 2003).

Effect of Maternal MeHg on Brain Development
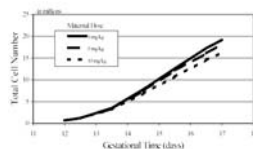
Findings showed that increasing doses of MeHg to the dam resulted in an increased deficit of neuronal cells at the end of midbrain development (i..e., 17 days). These could be compared to a critical value (e.g., a 10% reduction in final cell number from the untreated cells) to gauge the likelihood of brain malformation. Using a 10% criterion, the findings suggest that a single dose of 10 mg/kg MeHg would result in adverse developmental effects, while 5 mg/kg would not.

The effects of three different MeHg exposure patterns on midbrain development (i.e., one dose of 10 mg/kg given on GD 12; three doses of 3.3 mg/kg given on GD 12, 14 and 16; or one dose of 10 mg/kg on GD 15) were studied.

### Effect of Temporal MeHg on Brain Development
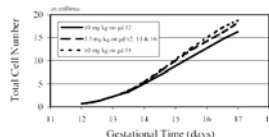
When given dose is spread over the midbrain developmental period (- - - -), the observed effect is less pronounced, while the effect of dosing near the end of the developmental period (. . . .) is minimal, with the total number of cells similar to the control population. This suggests that the alterations in cell division early in midbrain development have a greater impact than the alterations at later gestational time points. In addition, spreading out the total 10 mg/kg dose over the midbrain developmental period resulted in less severe depression in total midbrain cell number than a single dose given on GD 12.

EtOH Case Study

The developmental linked TK/TD model for EtOH focused on neocortical development because this region is exceptionally sensitive to this chemical during the prenatal period (i..e., second trimester) of neurogenesis. Thus, dose-response data for EtOH impact on neurogenesis versus apoptosis were incorporated into the Leroux model to evaluate neocortical development in rats and mice.

Impact of Maternal BEC on Fetus
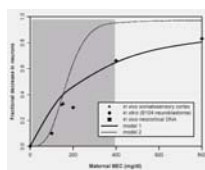
For comparison in this figure, (•) indicates in vivo data, (#) is DNA, and (—) is in vitro data. The grayed-in area indicates bounds of human blood ethanol concentrations (BEC) causing significant reductions in Bayley scores in 18-month old infants at one end (estimated from reported 0.5 oz of absolute alcohol/day) and death by respiratory arrest at the other. The two simulations (i.e., Models 1 and 2) correspond to the two different dose-response functions applied to the progenitor cells division rate.

Results suggested that 3 -5 drinks of alcohol a day by a pregnant woman would result in approximately 30% deficit in neocortical neurons due to the lengthening of the cell cycle. Preliminary simulations from this model suggested that at biologically relevant concentrations of EtOH (i.e., lower doses), the observed effects on neurogenesis versus apoptosis dominated the predicted neuronal cell impacts.

Previous studies have reported that in utero EtOH exposure resulted in a peak BEC of about 150 mg/dl (equivalent to a human female BEC after 3-5 drinks). This concentration increased the cell cycle length by about 6 hours in the ventricular region of rat fetus. In this study, cell cycle kinetic data from embryonic day 11 to 18 were used to estimate ventricular proliferation, and as inputs for TK/TD model simulations.

EtOH Exposure and Cellular Loss in Rats
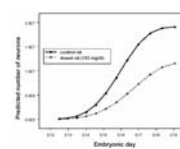
The TK/TD model was applied in this study to evaluate the potential effects of EtOH on apoptosis. The rich data-base for EtOH developmental neurotoxicity was used to validate a biologically based model in which cellular loss is the mode of action (MOA) for neurotoxicity. Ethanol-induced developmental cortical neurotoxicity is characterized by a range of cellular effects depending on the dose and time of exposure. However, cellular loss may be used to describe the key sensitive toxic effects of EtOH for risk assessment purposes.

Results/Conclusions

- The physiologically based TK/TD model was able to predict potential impacts from MeHg exposures that were consistent with results reported for in vivo teratology studies. Hence, the use of in vivo data confirmed that the model results derived solely from in vitro data.
- The MeHg findings indicated a concordance across mice, rats, an humans for effects on neuronal cell proliferation. In vivo data comparisons suggested that humans are probably more sensitive than rodents, and that mice may be a better model for assessing developmental toxicity.
- At human BEC after 3-5 drinks (~150 mg/dl), the EtOH model predicted a 25-30% neocortical cellular deficit by the end of neurogenesis in the rat.
- Preliminary model simulations suggested that at biologically relevant doses of EtOH, the observed effects on cell proliferation compared to apoptosis dominated the predicted neuronal cell impacts.

Impact and Outcomes

- The case studies in this research have identified the need for kinetic and dynamic models of specific lifestages. Understanding the kinetics and dynamics of neurodevelopmental impacts in one species can help identify the potential dose ranges, time, and target tissue in humans.
- This research has also identified critical data needs for health risk assessment. In particular, it has helped to define and prioritize data needs for developing and linking physiologically based TK/TD models, and understanding potential mechanisms of neurodevelopmental toxicity.
- The linked TK/TD modeling approach involves the parameterization and quantitation of specific physiologic processes. Hence, this will improve the risk assessments of sensitive subpopulations when data are extrapolated from animals to humans, and from adults to children.
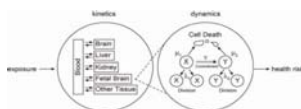
References

Gohlke, J.M. et al. (2002) A computational model for neocortical neurogenesis predicts ethanol-induced neocortical neuron number deficits. Develop. Neurosci. 24(6):467-477.

Gohlke, JM; et al. (2003) Evaluation of interspecies variability during neocortical neurogenesis using biologically based computational models. Toxicologist 72(S-1):37.

Leroux, BG; W. Leisenring, S. Moolgavkar, and E.M. Faustman (1996). A biologically based dose-response model for developmental toxicology. Risk Anal. 16(4):449–458.

Lewandowski, TA; et al. (2002) Methylmercury distribution in the pregnant rat and embryo during early midbrain organogenesis. Teratology 66(5):235–241.

Lewandowski, TA; et al. (2003) Effect of methylmercury on midbrain cell proliferation during organogenesis: potential cross-species differences and implications for risk assessment. Toxicol. Sci. 75(1):124–133.